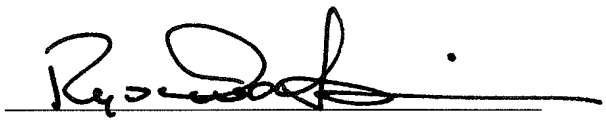
VERIFICATION OF TRANSLATION

I, Ryo Iwatani, of 15-15, Kamikotoen 3-chome, Nishinomiya-shi, HYOGO 662-0813 JAPAN, state the following:

I am fluent in both the English and Japanese languages and capable of translating documents from one into the other of these languages.

The attached document is a true and accurate English translation to the best of my knowledge and belief of the description, claims, drawings and abstract of Japanese Patent Application No. 2002-002056.

I state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signature: 

Ryo Iwatani

Date: May 14, 2009

[Name of Document] Patent application
[Reference Number] DS07J617
[Filing Date] January 9, 2002
[Addressee] To the Commissioner of the JPO
[Int. Cl.] C12N 9/10
[Inventor]
[Address or domicile] 201, 19-32, Uenohigashi 2-chome, Toyonaka-shi, OSAKA
[Name] TANIGUCHI Naoyuki
[Inventor]
[Address or domicile] 212, 6-17, Kamishinden 3-chome, Toyonaka-shi, OSAKA
[Name] MIYOSHI Eiji
[Inventor]
[Address or domicile] 306, 2-8, Onoharahigashi 5-chome, Minoo-shi, OSAKA
[Name] SAITO Takashi
[Applicant]
[Identification No.] 000001904
[Name or appellation] SUNTORY LIMITED
[Agent]
[Identification No.] 100077012
[Patent Attorney]
[Name or appellation] IWATANI Ryo
[Telephone Number] 06-4796-1300
[Indication of Fee]
[Deposit Account Number] 066372
[Fee(yen)] 21000
[List of Annexed Document]
[Name of matter] Specification 1
[Name of matter] Drawings 1
[Name of matter] Abstract 1

[Name of Document] Specification

[Title of the Invention]

GLYCOSYLTRANSFERASE GnT-V HAVING NEOVASCULARIZATION ACTION

[Scope of Patent Claims]

[Claim 1] A peptide or protein having a neovascularization action and containing a basic amino acid cluster region of β1,6-N-acetylglucosaminyltransferase.

[Claim 2] The peptide or protein according to Claim 1, wherein the β1,6-N-acetylglucosaminyltransferase has the following properties:

(1) Action: N-acetylglucosamine is transferred into α-6-D-mannoside using UDP-N-acetylglucosamine as a donor substrate;

(2) Substrate specificity: If the substrate specificity when GnGn-bi-PA is a receptor is 100%, the substrate specificity when GnGnF-bi-PA is a receptor is about 78%, the substrate specificity when GnGnGn-tri-PA is a receptor is about 125%, and the substrate specificity when GnM-PA is a receptor is about 66%;

(3) Optimum pH: 6.2 to 6.3;

(4) Activity: Mn^{2+} is not necessary for exertion of activity, and activity is not inhibited even in the presence of 20 mM EDTA;

(5) Molecular weight: About 73,000 (by SDS-PAGE in the absence of a reducing agent) and about 73,000 and about 60,000 (by SDS-PAGE in the presence of a reducing agent);

(6) Km value: Km values for a receptor GnGn-bi-PA and a donor UDP-GlcNAc are 133 μM and 3.5 mM, respectively;

(7) having the following peptide fragments:

(a) Thr-Pro-Trp-Gly-Lys,

(b) Asn-Ile-Pro-Ser-Tyr-Val,

(c) Val-Leu-Asp-Ser-Phe-Gly-Thr-Glu-Pro-Glu-Phe-Asn-His-Ala-Asn-Tyr-Ala,

(d) Asp-Leu-Gln-Phe-Leu-Leu, and

(e) Asn-Thr-Asp-Phe-Phe-Ile-Gly.

[Claim 3] The peptide or protein according to Claim 1, wherein the β1,6-N-acetylglucosaminyltransferase has an amino acid sequence containing at least the amino acid sequence as depicted in SEQ ID NO: 6, or an amino acid sequence obtained by modification of one or more amino acids in this amino acid sequence.

[Claim 4] The peptide or protein according to Claim 1, wherein, in the basic amino acid cluster region, the number of basic amino acids accounts for 30% or more of the total number of amino acids in said region.

[Claim 5] The peptide or protein according to Claim 1, wherein the basic amino acid cluster region has an amino acid sequence containing at least the amino acid sequence as depicted in SEQ ID NO: 7 or an amino acid sequence obtained by modification of one or more amino acids in this amino acid sequence.

[Claim 6] A neovascularization accelerator containing the peptide or protein according to any one of Claims 1 to 5.

[Claim 7] The neovascularization accelerator according to Claim 6, wherein the accelerator is a wound healing agent or a preventing and/or therapeutic agent for arteriosclerosis.

[Claim 8] A neovascularization inhibitor screening method, which comprises using the peptide or protein according to any one of Claims 1 to 5.

[Claim 9] A compound showing a neovascularization inhibiting action in the screening method according to Claim 8.

[Claim 10] A neovascularization inhibitor comprising the compound according to Claim 9.

[Claim 11] A neovascularization inhibitor screening method, which comprises using a protease cutting a mature type β1,6-N-acetylglucosaminyltransferase anchored on a Golgi body membrane to convert this into a secretory type β1,6-N-acetylglucosaminyltransferase.

[Claim 12] The screening method according to Claim 11, wherein the protease is β-secretase.

[Claim 13] A compound showing a neovascularization inhibiting action in the screening method according to Claim 11 or 12.

[Claim 14] A neovascularization inhibitor comprising the

compound according to Claim 13.

[Claim 15] A compound showing a neovascularization inhibiting action, characterized in that the compound suppresses expression of the peptide or protein according to any one of Claims 1 to 5.

[Claim 16] A neovascularization inhibitor comprising the compound according to Claim 15.

[Claim 17] A compound showing a neovascularization inhibiting action, characterized in that the compound suppresses binding of the peptide or protein according to any one of Claims 1 to 5 to heparan sulfate proteoglycan.

[Claim 18] A neovascularization inhibitor comprising the compound according to Claim 17.

[Claim 19] An antibody to the peptide or protein according to any one of Claims 1 to 5.

[Claim 20] An assay method for the peptide or protein according to any one of Claims 1 to 5, which comprises using the antibody according to Claim 19.

[Claim 21] A detection kit for the peptide or protein according to any one of Claims 1 to 5, which comprises the antibody according to Claim 19.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to a secretory type glycosyltransferase, neovascularization action of N-acetylglucosaminyltransferase V (hereinafter, abbreviated as GnT-V), a basic amino acid cluster of the GnT-V relating to the neovascularization action, use of GnT-V as neovascularization accelerator, a method of screening for an inhibitor of GnT-V and the basic amino acid cluster of GnT-V, a substance obtained by this screening method, a method of screening for a substance inhibiting production of secretory type GnT-V, a substance obtained by this screening method, and use of this substance as a neovascularization inhibitor.

[0002]

[Background Art]

In growth of cancers, factors such as fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) and the like are involved. Production of these factors and cytokines is controlled by complicated mechanisms such as increase in gene expression, modification after translation of gene products, mutual action with extracellular matrix, and so on.

[0003]

Many growth factors and receptors thereof are glycoproteins, and some of them are involved in neovascularization in tumor tissue. Recent studies using glycosyltransferase genes have revealed that change in the structure of an oligosaccharide of a growth factor receptor causes variation of intracellular

signal transduction, leading to cancerization of cells (Yamashita, K., et al., J. Biol. Chem. 260, 3963-3969 (1985). Pierce, M & Arango, J., J. Biol. Chem. 261, 10772-10777 (1986). Zhu, T.Y., et al., J. Cancer Res. Clin. Oncol. 123, 296-299 (1997). Petretti, T., et al., Gut 46, 359-366 (2000)). It is suggested that β1,6-N-acetylglucosaminyltransferase V (GnT-V) catalyzing formation of β(1,6) branch of asparagine sugar chain is the most important glycosyltransferase involved in metastasis of cancers (Demetriou, M., et al., J. Cell Biol. 130, 383-392 (1995). Dennis, J.W., et al., Science 236, 582-585 (1987)).

Neovascularization is an essential stage in progress of cancers such as metastasis and growth of cancers (Folkman, J., N. Eng. J. Med. 285, 1182-1186 (1971). Folkman, J. Ann. Surg. 175, 409-416 (1972)). A recent study using transgenic mice lacking GnT-V directly showed that GnT-V is essential for the growth of cancers and metastasis of cancers (Granovsky, M., et al., Nature Med. 6, 306-312 (2000)). Clinical studies have indicated increase in GnT-V activity in malignant tumors in lung and liver. It is shown that, in human lung cancer cells, GnT-V activity and size of tumors have a positive correlation (Dennis, J.W. & Laferte, S., Cancer Res. 49, 945-950 (1989)), and it is clarified that expression of GnT-V in human colon cancer cells is related with poor prognosis and metastasis (Murata, K., et al., Clin. Cancer Res. 6, 1772-1777 (2000)). However, detailed mechanisms of growth and metastasis of cancers via GnT-V have not been clarified yet.

[0004]

Asparagine type sugar chains (Asn type sugar chains) found

in glycoproteins are classified into three types depending on their constituent sugars and type of branching: high mannose type, complex type and hybrid type. Biosynthesis of these Asn type sugar chains initiates with one time transfer of sugar chain moieties from a lipid intermediate into asparagine of a polypeptide chain under translation, in the lumen side of rough endoplasmic reticulum. Thereafter, glucoses and some mannoses are removed in rough endoplasmic reticulum, but some glycoproteins having an Asn type sugar chain localized in rough endoplasmic reticulum stay there and keep high mannose type sugar chains. Other organelle glycoproteins, cell surface glycoproteins or secretory glycoproteins move to a Golgi body by vesicle transportation, and mannose is further removed. In this Golgi body, N-acetylglucosamine is introduced by the action of N-acetylglucosaminyltransferase groups which are Golgi body enzymes to give a branch structure. By formation of this branch structure, conversion from a high mannose type sugar chain into a hybrid type sugar chain and a complex type sugar chain initiates, and following introduction of fucose and introduction of galactose in a trans-Golgi region, finally, sialic acid is introduced to complete biosynthesis of Asn type sugar chains.

[0005]

It is known that various enzymes act as a catalyst in each step of the sequential Asn type sugar chain synthesis. Among them, six N-acetylglucosaminyltransferases are known as enzymes catalyzing a transfer and introduction reaction of N-acetylglucosamine in the formation of various branch structures of Asn type sugar chains. Schachter et al.

(Brockhausen, I., et al., Biochem. Cell Biol., 66, 1134(1988)) named these six enzymes transferring N-acetylglucosamine into a core structure of a trimannosyl structure of Man α1-3 (Man α1-6) Man β1-4 GlcNAc β1-4 GlcNAc as GnT-I to GnT-VI. Of them, GnT-V is an enzyme relating to formation of β(1,6) branch structure (-[GlcNAc β(1,6) Man α(1,6) Man]-). It is known that the β(1,6) branch structure is present in remarkably increased amount in cell transformation strains and tumor-forming cells (Pierce, M., et al., Biochem. Biophys. Res. Commun., 146, 679-684(1987) and Arango, J., & Pierce, M., J., Cell. Biochem., 257, 13421-13427(1982)). Further, it is shown that there is a relation between cancer metastatic potential of tumor-forming cells and emergence of a β(1,6) branch (Hiraizumi, et al., A., Arch. Biochem. Biophys. 280, 9-19, (1990)). It is reported that in human, emergence of a β(1,6) branch is increased in 50% of biopsy samples of breast carcinoma (Dennis, J.W., & Laferte, S. Cancer Res. 49, 945-950, (1989)). It is known that in any cases, emergence of a β(1,6) branch structure is accompanied by increase in GnT-V activity. Thus, GnT-V is an enzyme which is important not only in catalysis of formation of a β(1,6) branch structure in sugar chain biosynthesis route but also in relation with ease of metastasis (metastatic potential) and malignancy of cancer cells.

[0006]

[Problem to be Solved by the Invention]

The present invention has been made in view of the above-mentioned conditions and an object thereof is to provide a new therapeutic target relating to cancer metastasis and growth which are most important problems in cancer therapy, and

a therapeutic agent, a screening method of finding therapeutic agents, an evaluation method and a diagnosis method by clarifying a role played by a glycosyltransferase GnT-V on cancer metastasis and growth. Also, the present invention provides a new therapeutic idea that inhibition of secretion or expression of GnT-V suppresses not only cancer metastasis but also neovascularization which is a factor relating to cancer enlargement at metastasis site by providing a new biochemical concept that GnT-V promotes cancer metastasis and neovascularization. Further, the present invention provides a new drug design target in various ischemic diseases resulting from blood circulation disorder caused by vascular damage and the like, if neovascularization is regarded as a positive factor.

[0007]

[Means for Solving the Problem]

The present inventors have found that GnT-V which is one of glycosyltransferases has an action of accelerating neovascularization which is an initial regulation stage in cancer metastasis and subsequent cancer growth, as a new function utterly different from the original function as a glycosyltransferase. Namely, secretory type GnT-V and recombinant GnT-V which is purified promote *in vitro* and *in vivo* neovascularization at physiological concentration. Further, the present inventors have confirmed that the amino acid sequence of GnT-V comprises a basic amino acid cluster region containing a high amount of basic amino acids and that the region shows an action of releasing a fibroblast growth factor (FGF-2) from heparan sulfate proteoglycan (HSPG) on the surface of cells

and in extracellular matrix. One embodiment of the present invention is a peptide or protein having an amino acid sequence of a basic amino acid cluster region of GnT-V, and a neovascularization accelerator containing this peptide or protein.

[0008]

The present inventors have found that a glycosyltransferase GnT-V and a peptide having an amino acid sequence of a basic amino acid cluster region of this glycosyltransferase (basic peptide) accelerate neovascularization by releasing FGF-2 from HSPG on cancer cell surface, thereby promoting cancer metastasis and growth. On the basis of these findings, the present invention provides a method of screening for a compound inhibiting neovascularization induced by GnT-V and the above-mentioned basic peptide, a compound obtained by said screening method, and a neovascularization inhibitor containing said compound. More specifically, the present invention provides a method of screening for the following substances, a compound obtained by said screening method, and a neovascularization inhibitor containing said compound.

(a) A substance inhibiting a neovascularization action induced by GnT-V and a basic peptide

(b) A substance which inhibits a protease cutting mature GnT-V present in a Golgi body to convert this into a secretory type GnT-V

(c) A substance inhibiting gene expression of GnT-V

(d) A substance inhibiting release of FGF-2 from heparan sulfate proteoglycan induced by GnT-V and a basic peptide

[0009]

Namely, the present invention relates to

(1) A peptide or protein having a neovascularization action and containing a basic amino acid cluster region of β1,6-N-acetylglucosaminyltransferase, and

(2) The peptide or protein according to (1), wherein the β1,6-N-acetylglucosaminyltransferase has the following properties:

(a) Action: N-acetylglucosamine is transferred into α-6-D-mannoside using UDP-N-acetylglucosamine as a donor substrate;

(b) Substrate specificity: If the substrate specificity when GnGn-bi-PA is a receptor is 100%, the substrate specificity when GnGnF-bi-PA is a receptor is about 78%, the substrate specificity when GnGnGn-tri-PA is a receptor is about 125%, and the substrate specificity when GnM-PA is a receptor is about 66%;

(c) Optimum pH: 6.2 to 6.3;

(d) Activity: Mn^{2+} is not necessary for exertion of activity, and activity is not inhibited even in the presence of 20 mM EDTA;

(e) Molecular weight: About 73,000 (by SDS-PAGE in the absence of a reducing agent) and about 73,000 and about 60,000 (by SDS-PAGE in the presence of a reducing agent);

(f) Km value: Km values for a receptor GnGn-bi-PA and a donor UDP-GlcNAc are 133 μM and 3.5 mM, respectively;

(g) having the following peptide fragments:

(i) Thr-Pro-Trp-Gly-Lys (SEQ ID NO: 1),

(ii) Asn-Ile-Pro-Ser-Tyr-Val (SEQ ID NO: 2),

(iii) Val-Leu-Asp-Ser-Phe-Gly-Thr-Glu-Pro-Glu-Phe-Asn-His-Ala-Asn-Tyr-Ala (SEQ ID NO: 3),

(iv) Asp-Leu-Gln-Phe-Leu-Leu (SEQ ID NO: 4), and

(v) Asn-Thr-Asp-Phe-Phe-Ile-Gly (SEQ ID NO: 5).

[0010]

The present invention also relates to

(3) The peptide or protein according to (1), wherein the β1,6-N-acetylglucosaminyltransferase has an amino acid sequence containing at least the amino acid sequence as depicted in SEQ ID NO: 6, or an amino acid sequence obtained by modification of one or more amino acids in this amino acid sequence,

(4) The peptide or protein according to (1), wherein, in the basic amino acid cluster region, the number of basic amino acids accounts for 30% or more of the total number of amino acids in said region, and

(5) The peptide or protein according to (1), wherein the basic amino acid cluster region has an amino acid sequence containing at least the amino acid sequence as depicted in SEQ ID NO: 7, or an amino acid sequence obtained by modification of one or more amino acids in this amino acid sequence.

[0011]

The present invention also relates to

(6) A neovascularization accelerator containing the peptide or protein according to any of (1) to (5),

(7) The neovascularization accelerator according to (6), wherein the accelerator is a wound healing agent, or a preventing and/or therapeutic agent for arteriosclerosis,

(8) A neovascularization inhibitor screening method, which comprises using the peptide or protein according to any of (1) to (5),

(9) A compound showing a neovascularization inhibiting action in the screening method according to (8), and

(10) A neovascularization inhibitor comprising the compound according to (9).

[0012]

The present invention also relates to

(11) A neovascularization inhibitor screening method, which comprises using a protease cutting a mature type β1,6-N-acetylglucosaminyltransferase anchored on a Golgi body membrane to convert this into a secretory type β1,6-N-acetylglucosaminyltransferase,

(12) The screening method according to (11), wherein the protease is β-secretase,

(13) A compound showing a neovascularization inhibiting action in the screening method according to (11) or (12), and

(14) A neovascularization inhibitor comprising the compound according to (13).

[0013]

The present invention also relates to

(15) A compound showing a neovascularization inhibiting action, characterized in that the compound suppresses expression of the peptide or protein according to any of (1) to (5),

(16) A neovascularization inhibitor comprising the compound according to (15),

(17) A compound showing a neovascularization inhibiting action, characterized in that the compound suppresses binding of the peptide or protein according to any of (1) to (5) to heparan sulfate proteoglycan,

(18) A neovascularization inhibitor comprising the compound according to (17),

(19) An antibody to the peptide or protein according to any of (1) to (5),

(20) An assay method for the peptide or protein according to any of (1) to (5), which comprises using the antibody according to (19), and

(21) A detection kit for the peptide or protein according to any of (1) to (5), which comprises the antibody according to (19).

[0014]

[Embodiments of the Invention]

The present invention provides a neovascularization accelerator containing a peptide or protein containing a basic amino acid cluster region of β1,6-N-acetylglucosaminyltransferase.

The above-mentioned β1,6-N-acetylglucosaminyltransferase may be a known substance, and it is, however, preferable that said enzyme has the following enzymological properties.

(a) Action: N-acetylglucosamine is transferred into α-6-D-mannoside from UDP-N-acetylglucosamine;

(b) Substrate specificity: If the substrate specificity when GnGn-bi-PA is a receptor is 100%, the substrate specificity when GnGnF-bi-PA is a receptor is about 78%, the substrate specificity when GnGnGn-tri-PA is a receptor is about 125%, and the substrate specificity when GnM-PA is a receptor is about 66%;

(c) Optimum pH: 6.2 to 6.3;

(d) Activity: Mn^{2+} is not necessary for exertion of activity,

and activity is not inhibited even in the presence of 20 mM EDTA;

(e) Molecular weight: About 73,000 (by SDS-PAGE in the absence of a reducing agent) and about 73,000 and about 60,000 (by SDS-PAGE in the presence of a reducing agent);

(f) Km value: Km values for a receptor GnGn-bi-PA and a donor UDP-GlcNAc are 133 μM and 3.5 mM, respectively;

(g) having the following peptide fragments:

(i) Thr-Pro-Trp-Gly-Lys (SEQ ID NO: 1),

(ii) Asn-Ile-Pro-Ser-Tyr-Val (SEQ ID NO: 2),

(iii) Val-Leu-Asp-Ser-Phe-Gly-Thr-Glu-Pro-Glu-Phe-Asn-His-Ala-Asn-Tyr-Ala (SEQ ID NO: 3),

(iv) Asp-Leu-Gln-Phe-Leu-Leu (SEQ ID NO: 4), and

(v) Asn-Thr-Asp-Phe-Phe-Ile-Gly (SEQ ID NO: 5).

[0015]

In the present invention, an enzyme involved in the formation of β(1,6) branch structure (-[GlcNAc-β(1,6) Man-α(1,6) Man]-) (hereinafter referred to as GnT-V) is preferably used as the above-mentioned β1,6-N-acetylglucosaminyltransferase. Particularly, it is preferable that the above-mentioned β1,6-N-acetylglucosaminyltransferase has an amino acid sequence containing at least the amino acid sequence as depicted in SEQ ID NO: 6, or an amino acid sequence obtained by modification of one or more amino acids in this amino acid sequence. It is more preferable that the above-mentioned enzyme has an amino acid sequence described in Nishikawa, et al., Biochem. Biophys. Res. Commun. 198: 318-327 (1994).

[0016]

The above-mentioned enzyme can be easily obtained by known

methods. For example, human origin GnT-V can be isolated and purified from a rat kidney by the method described in Shoreibah, M., et al., J. Biol. Chem. 267, 2920-2927, (1992). It can be isolated and purified from concentrated liquid of a protein-free culture supernatant of human lung cancer (small cell carcinoma) origin QG cells by the method described in Japanese Patent Application Laid-Open (JP-A) No. 6-197756. The human lung cancer (small cell carcinoma) origin QG cell is named Human lung carcinoma SBM331, and internationally deposited with National Institute of Advanced Industrial Science and Technology (AIST), International Patent Organism Depositary (IPOD) under an acceptance number FERM BP-3967 on August 18, 1992 based on Budapest Treaty.

[0017]

The peptide or protein contained in the neovascularization accelerator of the present invention contains a basic amino acid cluster region of the above-mentioned GnT-V. The above-mentioned basic amino acid cluster region of GnT-V indicates a region containing a high amount of basic amino acids in which the number of amino acids is from about 5 to 50, preferably from about 8 to 40, more preferably from about 10 to 30. In the above-mentioned basic amino acid cluster region, it is preferable that the number of basic amino acids accounts for about 30% or more, preferably from about 35 to 95%, more preferably from about 40 to 90% of the total number of amino acids in the above-mentioned region.

[0018]

More preferably, the above-mentioned basic amino acid cluster region contains at least the amino acid sequence as

depicted in SEQ ID NO: 7. The above-mentioned basic amino acid cluster region may also contain at least an amino acid sequence obtained by modification of one or more amino acids in the amino acid sequence as depicted in SEQ ID NO: 7. Specifically, various modification type basic amino acid cluster regions are listed such as a peptide which has been subjected to addition of one or more amino acids to the amino acid sequence as depicted in SEQ ID NO: 7, and still maintains a neovascularization action; a peptide which has been subjected to removal of one or more amino acids from the above-mentioned amino acid sequence, and still maintains a neovascularization action; a peptide which has been subjected to substitution of one or more amino acids in the above-mentioned amino acid sequence by other amino acids, and still maintains a neovascularization action; further a peptide which has been subjected to a combination of the above-mentioned amino acid addition modification, amino acid removal modification and amino acid substitution modification, and still maintains a neovascularization action; and the like. The number of amino acids subjected to the above-mentioned modification such as amino acid addition, removal and substitution is not particularly restricted, but determined depending on the object of the modification, and specifically, it is about 30% or less, preferably about 20% or less, more preferably about 10% or less of the number of amino acids in the basic amino acid cluster region. It is preferable that the above-mentioned amino acid modifications such as addition, removal and substitution are conducted on moieties other than basic amino acids.

[0019]

The neovascularization accelerator according to the present invention may be the above-mentioned peptide or protein itself which is an active ingredient, but it is usually produced by mixing this active ingredient with a pharmaceutically acceptable carrier by a method known per se [methods commonly used in the field of formulation technologies, for example, methods described in the Japanese Pharmacopoeia (for example, 13th edition) and the like]. The dosage form of the neovascularization inhibitor according to the present invention includes, for example, oral preparations such as tablets, capsules (including soft capsules, microcapsules), powders, granules, syrups and the like, and parenteral preparations such as injections (for example, subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection and the like), external preparations (for example, intranasal preparation, percutaneous preparation and ointment), suppositories (for example, rectal suppository, vaginal suppository and the like), pellets, drops, sustained-release preparations (for example, sustained-release microcapsule and the like) and the like. The neovascularization inhibitor according to the present invention is preferably a parenteral preparation.

[0020]

As the pharmaceutically acceptable carrier, various organic or inorganic carrier substances commonly used as materials in the formulation are used, and listed are excipients, lubricants, binders and disintegrating agents in solid preparations; and solvents, solubilizing agents, suspending agents, isotonizing agents, buffers, soothing agents and the

like in liquid preparations. If necessary, additives in the formulation such as preservatives, antioxidants, coloring agents, sweetening agents and the like can also be used.

[0021]

Preferable examples of the excipient include lactose, sucrose, D-mannitol, starch, crystalline cellulose, light silicic acid anhydride and the like. Preferable examples of the lubricant include magnesium stearate, calcium stearate, talc, colloidal silica and the like. Preferable examples of the binder include crystalline cellulose, sucrose, D-mannitol, dextrin, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpyrrolidone and the like. Preferable examples of the disintegrating agent include starch, carboxymethylcellulose, carboxymethylcellulose calcium, crosscarmellose sodium, carboxymethyl starch sodium and the like.

[0022]

Preferable examples of the solvent include water for injection, alcohol, propylene glycol, macrogol, sesame oil, corn oil and the like.

Preferable examples of the solubilizing agent include polyethylene glycol, propylene glycol, D-mannitol, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate and the like.

Preferable examples of the suspending agent include surfactants such as stearyltriethanolamine, sodium lauryl sulfate, laurylaminopropionic acid, lecithin, benzalkonium chloride, benzethonium chloride, glycerin monostearate and the like; and hydrophilic polymers such as polyvinyl alcohol,

polyvinylpyrrolidone, carboxymethylcellulose sodium, methylcellulose, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose and the like.

[0023]

Preferable examples of the isotonizing agent include sodium chloride, glycerin, D-mannitol and the like.

Preferable examples of the buffer include buffer solutions of phosphates, acetates, carbonates and citrates and the like.

Preferable examples of the soothing agent include benzyl alcohol and the like.

Preferable examples of the preservative include p-hydroxybenzoate ester, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid, sorbic acid and the like.

Preferable examples of the antioxidant include sulfite, ascorbic acid and the like.

[0024]

The neovascularization accelerator according to the present invention can be used for mammals (for example, human, mouse, rat, rabbit, dog, cat, bovine, horse, swine, monkey and the like).

The application of the neovascularization accelerator according to the present invention is not particularly restricted, but the neovascularization accelerator is preferably used as a wound healing agent. The dose of the agent in this case is not determined indiscriminately since it varies depending on the type of disease conditions to be treated, the age and body weight of patient, symptoms, the seriousness of disease and the like, but it is about 0.01 to 100 mg/kg,

preferably about 0.1 to 50 mg/kg. Particularly, when the neovascularization accelerator according to the present invention is locally applied, in the form of liquid, ointment, cream, gel, cataplasm and the like, to a wound region and absorbed percutaneously to heal the wound, the above-mentioned peptide or protein can be applied, for example, at a concentration of about 0.001 to 1000 mg/ml, further preferably of about 0.01 to 500 mg/ml.

[0025]

The neovascularization accelerator according to the present invention can be used for treatment or prevention of aneurysm; arteriosclerosis such as coronary arteriosclerosis, cerebral arteriosclerosis or peripheral arteriosclerosis; peripheral artery obstruction, acute myocardial infarction (AMI), deep-vein thrombosis, pulmonary embolism, dissecting aneurysm, transient ischemic attack (TIA), apoplexy, and other obstruction-related disorders; unstable angina pectoris, disseminated intravascular coagulation (DIC), sepsis, surgical or infectious shock, postoperative and postpartum trauma, cardiopulmonary bypass surgical operation, incompatible blood transfusion, premature separation of the placenta, thrombotic thrombocytopenia purpura (TTP), acute or chronic renal diseases due to excess agglomeration such as snake venom and immune diseases, inflammation, hemolytic-uremic syndrome, symmetric peripheral necrosis, and bedsore. Further, the neovascularization accelerator according to the present invention can be used for enhancing the action of a thrombolytic agent, preventing re-obstruction, preventing re-obstruction after PTCA, preventing thrombocytopenia resulting from

dialysis, and preventing thrombosis caused by artificial blood vessels and organs.

When the neovascularization accelerator according to the present invention is used in the above-mentioned applications, the dose thereof is not determined indiscriminately since it varies depending on the application of the neovascularization accelerator, the type of disease conditions to be treated, the age and body weight of patient, symptoms, the seriousness of disease and the like, but it is about 0.01 to 100 mg/kg, preferably about 0.1 to 50 mg/kg, per day. Particularly, when administered intravenously, the dose thereof is about 0.01 to 5 mg/kg, preferably about 0.04 to 1.5 mg/kg, per day. It is desirable that this dose is administered 1 to 3 times per day. [0026]

In the neovascularization accelerator of the present invention, there can be used a concomitant drug not giving any adverse effect on the neovascularization action of the peptide or protein according to the present invention. The concomitant drug is not particularly restricted and when the neovascularization accelerator of the present invention is used as a treating and/or preventing agent for arteriosclerosis and the like, examples thereof include hypotensive agents, hypolipidemic agents, diuretics, thrombolytics and the like.

The timing of administration of the neovascularization accelerator according to the present invention and concomitant drug is not particularly restricted and these may be administered simultaneously, or administered at a time interval, to the subject to be administered. The dose of the concomitant drug may be advantageously determined according to clinically

used dose, and can be appropriately selected depending on the target subject, age and body weight of the target subject, symptoms, time of administration, dosage form, administration route, combination and the like. The administration form of the concomitant drug is not particularly restricted, and it may be advantageous that the neovascularization accelerator according to the present invention and concomitant drug are combined at the time of administration.

[0027]

The present invention provides an antibody to a peptide or protein containing a basic amino acid cluster region of β1,6-N-acetylglucosaminyltransferase. The above-mentioned antibody to the peptide or protein as an antigen may be either of a monoclonal antibody and polyclonal antibody. These antibodies can be produced according to known methods described, for example, in "Basic Experiment Method of Protein and Enzyme, 2nd revision (T. Horio ed., published by NANKO DO, 1994)," "Method in Enzymology vol. 182 published by ACADEMIC PRESS, INC. 1990" and the like.

[0028]

The present invention provides an assay method for the above-mentioned peptide or protein having a neovascularization action using these antibodies, and a detection kit for the above-mentioned peptide or protein having a neovascularization action using this assay method. Such an assay method and detection kit can be utilized in various applications. For example, neovascularization is an essential process in cancer metastasis, and therefore, a possibility of cancer metastasis can be found by determining the presence or absence or the amount

of the above-mentioned peptide or protein having a neovascularization action in the blood or cancer tissue of a patient with cancer using the assay method and detection kit according to the present invention.

In the assay method and detection kit according to the present invention, an antibody molecule itself may be used, and alternatively, $F(ab')_2$, Fab' or Fab fractions of the antibody molecule may be used.

[0029]

For the above-mentioned assay method and production of detection kit, known methods can be used. For example, as the method for quantifying the above-mentioned peptide or protein having a neovascularization action using the above-mentioned antibody, there can be used any assay method in which the amount of an antibody corresponding to the antigen amount (for example, protein amount), or the amount of an antigen or an antibody-antigen complex in a test solution is detected by chemical or physical means, and calculated from the standard curve produced by using a standard solution containing an antigen in known amount. For example, nephrometry, competition method, immunometric method and sandwich method are suitably used, and it is particularly preferable to use a sandwich method described later from the standpoint of sensitivity and specificity.

[0030]

Specific embodiments of the assay method according to the present invention will be described below, but the scope of the invention is not limited to these embodiments. Namely, there is exemplified, as the above-mentioned assay method, (i) a

method of quantifying the above-mentioned peptide or protein having a neovascularization action in a test solution, in which an antibody to the above-mentioned peptide or protein having a neovascularization action, a test solution, and the above-mentioned peptide or protein having a neovascularization action which has been labeled (hereinafter, referred to as simply "labeled peptide" in this column) are allowed to react competitively, and the proportion of the labeled peptide bound to the antibody is measured. Then, there is also mentioned (ii) a method of quantifying a peptide or protein having a neovascularization action in a test solution in which; the antibody to the above-mentioned peptide or protein having a neovascularization action is held on a carrier to provide insolubility; meanwhile, another antibody to the peptide or protein having a neovascularization action, which recognizes a region other than what the above-mentioned insolubilized antibody recognizes, is labeled; next, the test solution, the antibody insolubilized on the carrier, and the labeled antibody are reacted simultaneously or sequentially; and then, the activity of the labeling agent trapped via the antigen (peptide or protein having a neovascularization action) on the carrier and/or the activity of the labeling agent not trapped on the carrier is measured. Further, as the method of assaying the peptide or protein having a neovascularization action of the present invention, detection by tissue staining and the like can also be conducted in addition to quantification of the peptide or protein having a neovascularization action using a monoclonal antibody to the peptide or protein.

[0031]

In an assay method involving the use of a labeled substance among such assay methods according to the present invention, for example, radioactive isotopes, enzymes, fluorescent substances, light-emitting substances and the like are used as a labeling agent. As the above-mentioned radioactive isotope, for example, ^{125}I, ^{131}I, ^{3}H, ^{14}C and the like are used. As the above-mentioned enzyme, those which are stable and have large specific activity are preferable, and examples thereof include β-galactosidase, β-glucosidase, alkaline phosphatase, peroxidase, malic acid dehydrogenase and the like. As the above-mentioned fluorescent substance, for example, fluorescamine, fluorescein isothiocyanate and the like are used. As the above-mentioned light-emitting substance, for example, luminol, luminol derivatives, luciferin, lucigenin and the like are used. Furthermore, a biotin-avidin system can also be used for binding of an antibody or antigen with a labeling agent.
[0032]

The present invention provides a neovascularization inhibitor. The neovascularization inhibitor according to the present invention is characterized in that it comprises one or more compounds selected from the group consisting of (a) a compound showing a neovascularization inhibiting action in the above-mentioned screening method using a peptide or protein having a neovascularization action, (b) a compound inhibiting the activity of a protease cutting a mature type β1,6-N-acetylglucosaminyltransferase anchored on a Golgi body membrane to convert this into a secretory type β1,6-N-acetylglucosaminyltransferase, (c) a compound suppressing expression of the above-mentioned peptide or

protein having a neovascularization action, and (d) a compound suppressing binding of the above-mentioned peptide or protein having a neovascularization action to heparan sulfate proteoglycan. The above-mentioned compounds (a) to (d) will be described in detail below.

[0033]

The above-mentioned compound (a) showing a neovascularization inhibiting action in a screening method using a peptide or protein having a neovascularization action can be obtained by a screening method as described below. Namely, as this screening method, there is mentioned a method in which neovascularization is observed in the case of the presence of the above-mentioned peptide or protein having a neovascularization action and a test substance in a system of observing neovascularization described in detail in examples, and it is compared with neovascularization in the case of the absence of the test substance. When neovascularization in the case of the presence of a test substance is less as compared with neovascularization in the case of the absence of the test substance in such a screening method, such a test substance is recognized as a substance showing a neovascularization inhibiting action. More specifically, it may be advantageous that the total length of newly-produced blood vessels measurable from a micrograph in the case of the presence of a test substance is about 90% or less, preferably about 80% or less, more preferably about 70% or less, based on that in the case of the absence of the test substance.

[0034]

Here, the test substance used in the above-mentioned

screening method is not particularly restricted, and may be a protein, a compound of low molecular weight, or a compound of high molecular weight. It may also be a purified substance, or a mixture containing several co-existent compounds. Further, it may also be that of natural origin such as culture solution of a microorganism or a chemically synthesized substance. Moreover, the test substance may be a novel compound or a known compound. With respect to these descriptions, the same shall be applied also in the following screening method.
[0035]

The compound (b) inhibiting the activity of a protease cutting a mature type GnT-V anchored on a Golgi body membrane to release this from a Golgi body membrane and to convert this into a secretory type GnT-V can be easily obtained by a screening method using the above-mentioned protease. The screening method using the above-mentioned protease is not particularly restricted, and when the secretory type GnT-V generated by allowing the above-mentioned protease to act on the mature type GnT-V anchored on a Golgi body membrane in the presence of a test substance is smaller in amount as compared with that obtained in the absence of the test substance, such a test substance is recognized as a compound inhibiting the activity of a protease. Screening for the above-mentioned test substance inhibiting the activity of a protease may also be performed in a test tube.

As the above-mentioned protease, for example, β-secretase and the like are listed. The amino acid sequence of the β-secretase is described in Vassar, R., et al., Science 286, 735-741(1999) and the like, and easily available from

information of GenBank accession number AF190725 and the like.

[0036]

The compound (c) suppressing expression of the above-mentioned peptide or protein having a neovascularization action can be obtained by known methods. For example, a method using a promoter for expression of the above-mentioned peptide or protein having a neovascularization action and a reporter gene (T. Yokota, K. Arai, Biomanual series 4, YODO sha (1993)) is mentioned. More specifically, the above-mentioned promoter is connected to a translation region of the reporter gene to produce an expression vector, this expression vector is introduced into a host cell to produce a transformant, this transformant is cultured for a certain time, then, any amount of a test substance is added, and the amount of the reporter expressed in the cell after a certain time is measured as an enzyme activity, or as the amount of the expressed protein. More specifically, when the expression amount of the reporter gene in the presence of a test substance is smaller as compared with the expression amount of the reporter gene in the absence of the test substance, such a test substance can be recognized as a substance suppressing expression of the above-mentioned peptide or protein having a neovascularization action.

[0037]

In the above-mentioned method, it is preferable to use a promoter region upstream of a GnT-V gene as the promoter for expression of the above-mentioned peptide or protein having a neovascularization action. Such a promoter can be obtained by cloning 5'-upstream region of a GnT-V gene from the genome of an HuCC-T1 cell (Saito, H., et al., Eur. J. Biochem. 233, 18-26

(1995)). The HuCC-T1 cell can be obtained from Japanese Cancer Resources Bank.

In the above-mentioned method, any genes encoding peptides or proteins may be used as the reporter gene so long as the activity or production amount of the expressed product (also including the production amount of mRNA) can be measured by persons skilled in the art. For example, chloramphenicol acetyltransferase (CAT), β-glactosidase (β-Gal), luciferase and the like can be utilized by measuring their enzymatic activities. Secretory type growth hormone and the like can be utilized by measuring its production amount by an immune antibody reaction method and the like.

[0038]

The above-mentioned expression vector can be obtained by inserting a translation region of the above-mentioned promoter and reporter gene into a replicable vector. The replicable vector is not particularly restricted, and pUC18, pGEM-3Z and the like are listed as a vector replicable in *E. coli*. The above-mentioned expression vector is introduced into a host cell to produce a transformant. The host cell is not particularly restricted and can be selected appropriately depending on the type of the expression vector. Such transformation can be conducted by usual methods. As the transformant used in the present invention, those in which an expression vector is transiently introduced into a host are also used, in addition to those in which an expression vector is stably introduced into a host chromosome. Selection of the transformants in which an expression vector is stably introduced into a host chromosome can be conducted by

transforming a host cell with the expression vector of interest into which a selection marker gene has been also introduced, or with both a vector containing a selection marker and the expression vector of interest, and culturing the transformed cell in a medium in which only a vector having a selection marker can survive.

[0039]

More preferably, a compound suppressing expression of the above-mentioned peptide or protein having a neovascularization action can be obtained by the following method. Namely, (a) DNA containing 5'-GGGAGTGAGGATGATGTAGGGAAG-3' (SEQ ID NO: 8), 5'-ATGGGGCAGAGGAACTTACGTTAT-3' (SEQ ID NO: 9), or at least one of the above base sequences; (b) an Ets-1 protein or fragment thereof; and (c) a test substance are incubated together, and binding of the above-mentioned DNA (a) with an Ets-1 protein or fragment thereof is measured.

Transcription of a GnT-V gene is promoted by binding of an Ets-1 protein to a specific site shown by either of the above-mentioned sequences in an upstream promoter region of a GnT-V gene. The peptide or protein contains at least a basic amino acid cluster region of GnT-V. Therefore, a test substance inhibiting binding of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof can suppress expression of the above-mentioned peptide or protein having a neovascularization action.

[0040]

As the method of measuring binding of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof, known methods may be used. As preferable embodiments of such a

measuring method, a gel shift assay and supershift assay are listed and these methods will be illustrated in detail below.

[0041]

The gel shift assay is conducted, for example, as described below. 5'-extended terminal of the above-mentioned DNA sequence is labeled using [γ-^{32}P] dATP (available from Amersham). The resulted ^{32}P labeled DNA (10,000 cpm) and a cut Ets-1 protein or nuclear extract obtained from MOLT4 cells in which the Ets-1 protein is *in vitro* transcribed/translated are mixed together with a buffer containing 65 mM KCl, 25 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 0.25 mM EDTA and 10 % glycerol so that the total volume is 20 ml. Subsequently, 2 μg of poly(dI-dC) (available from Sigma) is added to the reaction mixture. Then, the reaction mixture is cultured for 1 hour at room temperature. The resulted culture solution is applied on 6% non-denaturing polyacrylamide gel (acrylamide:bisacrylamide = 29:1) in 0.5 × TBE (1 × TBE = 89 mM Tris, 89 mM boric acid, 2 mM EDTA), and then, electrophoresis is conducted at 4°C and 150 V for 1 hour. After electrophoresis, the gel is dried by a gel drier, and then, exposed to an X-ray film (available from Kodak).

[0042]

In the gel shift assay, the mobility manifested by the complex of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof in electrophoresis using non-denaturing polyacrylamide gel decreases as compared with that manifested by the above-mentioned DNA sequence not bound to an Ets-1 protein or fragment thereof. When a test substance is added in given amount to the above-mentioned reaction mixture, if a band of a complex of the above-mentioned DNA sequence with

an Ets-1 protein or fragment thereof is not observed or its band quantity decreases in the result of electrophoresis obtained by the above-mentioned procedure, the test substance can be judged to be a substance inhibiting binding of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof.

[0043]

The supershift assay is conducted in the same manner as the gel shift assay, except that anti-Ets-1 IgG (available from Cambridge Research Biochemicals) not cross-reacting with a protein in other Ets family is added to the reaction mixture. In the supershift assay, the mobility manifested by the complex of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof in electrophoresis using non-denaturing polyacrylamide gel decreases as compared with that manifested by the above-mentioned DNA sequence not bound to an Ets-1 protein or fragment thereof, to a greater extent than in the gel shift assay. When a test substance is added in given amount to the above-mentioned reaction mixture, if a band of a complex of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof is not observed or its band quantity decreases in the result of electrophoresis obtained by the above-mentioned procedure, the test substance can be judged to be a substance inhibiting binding of the above-mentioned DNA sequence with an Ets-1 protein or fragment thereof.

[0044]

The compound (d) suppressing binding of the above-mentioned peptide or protein having a neovascularization action to heparan sulfate proteoglycan may also be a compound which

decreases affinity of the above-mentioned peptide or protein having a neovascularization action with heparan sulfate proteoglycan, as well as a compound preventing binding of the above-mentioned peptide or protein having a neovascularization action to heparan sulfate proteoglycan. The above-mentioned peptide or protein having a neovascularization action binds to heparan sulfate proteoglycan on the surface of a cell or on an extracellular matrix in competition with FGF-2 (fibroblast growth factor-2). Further, since the above-mentioned peptide or protein having a neovascularization action has higher affinity, than FGF-2, with heparan sulfate proteoglycan, FGF-2 bound to heparan sulfate proteoglycan is dissociated from heparan sulfate proteoglycan. Thus generated free FGF-2 stimulates endothelial cells to cause neovascularization. Therefore, in the presence of a compound which prevents binding of the above-mentioned peptide or protein having a neovascularization action to heparan sulfate proteoglycan or which decreases affinity thereof with heparan sulfate proteoglycan, FGF-2 can bind dominantly to heparan sulfate proteoglycan and the process of neovascularization as described above does not progress.

[0045]

As the compound suppressing binding of the above-mentioned peptide or protein having a neovascularization action to heparan sulfate proteoglycan, for example, compounds blocking a basic amino acid cluster region of this peptide or protein, and the like are listed. Specific examples of such compounds include peptides and proteins containing an acidic amino acid cluster region containing a high amount of acidic amino acids.

Preferable as the above-mentioned acidic amino acid cluster region is a region containing a high amount of acidic amino acids in which the number of amino acids of about 5 to 50, preferably about 8 to 40, more preferably about 10 to 30. In the above-mentioned acidic cluster region, it is preferable that the number of acidic amino acids accounts for about 30% or more, preferably about 35 to 95%, more preferably about 40 to 90% of the total number of amino acids in the above-mentioned region.
[0046]

The neovascularization inhibitor according to the present invention may be at least one compound itself among the above-mentioned compounds (a) to (d) each of which is an active ingredient, but it is usually produced by mixing the active ingredient with a pharmaceutically acceptable carrier by a method known per se [methods commonly used in the field of formulation technologies, for example, methods described in the Japanese Pharmacopoeia (for example, 13th edition), and the like]. The dosage form of the neovascularization inhibitor according to the present invention includes, for example, oral preparations such as tablets, capsules (including soft capsules, microcapsules), powders, granules, syrups and the like, and parenteral preparations such as injections (for example, subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection and the like), external preparations (for example, intranasal preparation, percutaneous preparation and ointment), suppositories (for example, rectal suppository, vaginal suppository and the like), pellets, drops, sustained-release preparations (for example, sustained-release microcapsule and the like) and the like.

Particularly, the neovascularization inhibitor according to the present invention is preferably a parenteral preparation. Here, as the pharmaceutically acceptable carrier, the above-mentioned compounds are listed.

[0047]

The neovascularization inhibitor according to the present invention can be used for mammals (for example, human, mouse, rat, rabbit, dog, cat, bovine, horse, swine, monkey and the like). The dose thereof is not determined indiscriminately since it varies depending on the type of active ingredients of neovascularization inhibitors, the type of disease conditions to be treated, the age and body weight of patients, symptoms, the seriousness of diseases, and the like.

[0048]

The application of the neovascularization inhibitor according to the present invention is not particularly restricted and the neovascularization inhibitor can be used as a preventing and treating agent for various diseases accompanied by neovascularization, for example, tumors (for example, malignant melanoma, malignant lymphoma, gastrointestinal (e.g., stomach, intestine and the like) cancer, lung cancer, pancreas cancer, esophageal cancer, breast cancer, liver cancer, ovarian cancer, uterine cancer, prostate cancer, kidney cancer, bladder cancer, brain cancer, Kaposi's sarcoma, angioma, osteosarcoma, myosarcoma, angiofibroma and the like), inflammatory diseases (for example, rheumatic arthritis, psoriasis and the like), diabetic retinopathy, atherosclerosis (including abnormal angiopoiesis in the formation of abnormal capillary network in atherosclerosis nest outer membrane) and

the like. The neovascularization inhibitor of the present invention can be used also as an agent for treating eye hyperemia.

[0049]

In the neovascularization inhibitor of the present invention, there can be used a concomitant drug not exerting any adverse effect on the neovascularization inhibiting action of the above-mentioned compounds (a) to (d). As such a concomitant drug, there are listed, for example, antitumor agents, cachexy improving agents, antidiabetic agents other than insulin resistant improving agents, diabetic complication treating agents, antiobestic agents, hypotensive agents, hypolipidemic agents, diuretics and the like, and two or more of them may be combined. In use of the neovascularization inhibitor of the present invention, surgical therapy (operation) or radiation therapy may be conducted.

[0050]

The timing of administration of the neovascularization inhibitor according to the present invention and concomitant drug is not particularly restricted and these may be administered simultaneously, or administered at a time interval, to the subject to be administered. The dose of the concomitant drug may be advantageously determined according to clinically used dose, and can be appropriately selected depending on the target subject, age and body weight of the target subject, symptoms, time of administration, dosage form, administration route, combination and the like. The administration form of the concomitant drug is not particularly restricted, and it may be advantageous that the neovascularization inhibitor

according to the present invention and concomitant drug are combined at the time of administration.

[0051]

[Examples]

The present invention will be illustrated in detail by the following examples, but the scope of the invention is not limited to these examples.

[0052]

[Example 1: Acceleration of neovascularization in a nude mouse by metastasis of GnT-V transformant]

Considering expression of GnT-V shows a high correlation with metastasis and poor prognosis of colon cancer, a transformant of β1,4-N-acetylglucosaminyltransferase III (GnT-III) or α1,6-fucosyltransferase (FucT) as a control was produced together with a stable transformant of GnT-V using a human colon cancer cell WiDr, and each of these transformants was injected subcutaneously to a nude mouse to examine an influence exerted on cancer metastasis. The human colon cancer cell strain WiDr was cultured on an RPMI-1640 medium (manufactured by GIBCO BRL) containing 10% fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin). Transformation was conducted using a CELL FECTIN (registered trademark) reagent (manufactured by GIBCO BRL) according to a method described in a manual of CELL FECTIN (registered trademark). Regarding transplantation of the above-mentioned transformed cancer cell to a nude mouse, 5×10^5 cells transformed with each of the above-mentioned glycosyltransferases were injected into the back of the nude mouse, and formation of cancer and neovascularization were

visually observed one month after. Though the WiDr cell originally contains slight expression of the above-mentioned glycosyltransferases, acceleration of cancer metastasis was observed and remarkable neovascularization was observed in tumor tissue in the nude mouse transplanted with the GnT-V transformant as compared with the nude mouse transplanted separately with WiDr cells transformed with the other respective glycosyltransferase genes. This result suggests that a cancer cell excessively expressing GnT-V secretes a certain factor accelerating neovascularization.

[0053]

[Example 2: Induction of neovascularization by GnT-V transformant]

Acceleration of neovascularization by a GnT-V transformant was confirmed by a CAM (chorioallantoic membrane) assay using an embryo of a chicken fertilized egg. The CAM assay was conducted by a method of Yen et al. (Yen, L., et al., Oncogene 19, 3460-3469 (2000)) and a method of Bernardini (Bernardini, G., et al., Blood 96, 4039-4045 (2000)), both being slightly modified. CAM of white Leghorn 8 days after fertilization was used, and 1×10^5 cells transformed with each of the above-mentioned glycosyltransferases were seeded on a collagen sponge and maintained for 4 hours. A 5 mm silicon ring was placed on the CAM on the collagen sponge, which was maintained for 48 hours. It was found that invading of blood vessels into the collagen sponge was observed only in the case of the GnT-V transformant among WiDr cells transformed with the respective glycosyltransferase genes described in Example 1. Also in the case of cells obtained by transient transformation of a GnT-V

gene into WiDr cells and noncancerous cells such as COS-1 cell and CHO cell, acceleration of neovascularization was observed in the CAM assay as in the case of the above-mentioned GnT-V stable transformant. These results strongly suggest that a common mechanism exists for acceleration of neovascularization by expression of GnT-V.

[0054]

[Example 3: Induction of neovascularization by culture solution of GnT-V transformant]

For *in vitro* evaluation of induction of neovascularization by a GnT-V transformant, the amount of synthesis of DNA of human umbilical vein epithelial cells (HUVEC) after stimulation with culture solution of a GnT-V transformant was measured by a method of Soker et al.(Soker, S., et al., J. Biol. Chem. 272, 31582-31588 (1997)). HUVEC was seeded on a 96-well plate coated with type I collagen in an amount of 2×10^3 cells per well, and 24 hours later, the medium was substituted with an MCDB131 medium (not containing FBS and FGF-2) containing 0.1% fetal bovine serum albumin and a starved condition was maintained for 24 hours. The medium was substituted with each culture solution of the WiDr cells transformed with the respective glycosyltransferase genes described in Example 1, and HUVEC was stimulated for 24 hours. HUVEC was maintained for 8 hours with [^{3}H]-thymidine (1 μCi/ml), and incorporation of [^{3}H]-thymidine into HUVEC was analyzed by MicroBeta-Counter (manufactured by Wallac) to measure the amount of synthesis of DNA. The result was shown as the mean value of assay results of 6 wells, and the standard deviation was measured. All experiments were repeated at least three times, and the same results were

obtained. As apparent from Fig. 1, DNA synthesis of HUVEC stimulated with the culture solution of WiDr cells transformed with a GnT-V gene increased, but the same effect was not observed in culture solution of WiDr cells transformed with the other respective glycosyltransferase genes. These results indicate that the WiDr cell transformed with a GnT-V gene secretes a neovascularization factor into culture solution as a result of excess expression of GnT-V.

[0055]

[Example 4: Influence of recombinant GnT-V on differentiation and growth of HUVEC]

Purification of a neovascularization factor present in culture solution of WiDr cells transformed with a GnT-V gene was conducted using various column chromatographies. The neovascularization activity of each fraction was evaluated as a measure of differentiation and growth of HUVEC as described in Example 3. In heparin affinity chromatography, a fraction of high growth activity for HUVEC was eluted with 0.3 M NaCl. Since known growth factors such as FGF-1, FGF-2, VEGF, placenta-induced growth factor (PIGF), hepatocyte growth factor (HGF) and the like are eluted with 0.8 to 1.5 M NaCl (Hauser, S. & Weich H.A., Growth Factor 9, 259-268 (1993). Gohda, E., et al., J. Clin. Invest. 81, 414-419 (1998). Marez, A., et al., Biochimie 69, 125-129 (1987). Risau, W., et al., The EMBO J. 7, 959-962 (1988). Rothenthal, R.A., et al., Growth Factor 4, 53-59 (1990)), the above-mentioned nature is utterly different from the natures of these known growth factors. The WiDr cell itself does not produce such a neovascularization factor. The fraction eluted with 0.3 M NaCl in heparin affinity

chromatography was subjected to Western blot analysis using an anti-GnT-V antibody. It was found that the reaction of the anti-GnT-V antibody and the differentiation and growth activity for HUVEC are consistent with each other, and the main protein having a differentiation and growth activity for HUVEC present in the fraction is GnT-V itself.

[0056]

Though it is known that GnT-V is secreted from cancer cells (Chen, L., et al., Glycoconjugate J. 12, 813-823 (1995)), like other glycosyltransferases (Gu, J., et al., J. Biochm. 113, 614-619 (1993). MaCaffery, G. & Jamison, J.C., Comp. Biochem. Physiol. B. 104, 91-94 (1993). Ugarte, M.A. & Rodriguez, P., J. Biochem. 23, 719-726 (1991)), the physiological significance of secretion of these glycosyltransferases is not known. For verifying a hypothesis that secretory type GnT-V itself induces differentiation and growth of HUVEC, a recombinant GnT-V, called GnT-VΔ73, maintaining a glycosyltransferase activity but lacking its transmembrane region was produced. The GnT-VΔ73 which is a soluble recombinant GnT-V was prepared by a Baculovirus system according to a method disclosed in a literature of Sasai et al (Sasai, K., et al., Glycobiology (in press)). As shown in Fig. 2, by addition of GnT-VΔ73, recombinant GnT-V, differentiation and growth of HUVEC increased in addition amount-dependent manner. The concentration of the used GnT-VΔ73 was in the physiological range, and the concentration of GnT-V present in culture solution of GnT-V transformants was 140 ng/ml based on the specific activity of GnT-VΔ73. A mouse melanoma cell B16-F10 had a high endogenous GnT-V activity, the culture solution of

B16-F10 cells contained 70 ng/ml of GnT-V, and also the B16-F10 cell showed the same neovascularization activity in the CAM assay. Addition of recombinant Fuc-T did not show HUVEC growth accelerating activity at all. These results show that secretory type GnT-V in the physiological concentration range has an HUVEC growth accelerating activity.

[0057]

[Example 5: Analysis of domain of GnT-V involved in differentiation and growth of HUVEC]

For clarifying which domain of GnT-V is involved in HUVEC growth accelerating activity, deletion variants of GnT-V shown in Fig.3A were produced. The method of production of a GnT-VΔ188 plasmid is disclosed in a literature of Sasai et al (Sasai, K., et al., Glycobiology (in press)). A transfer plasmid having a GnT-VΔ233 gene was produced by ligating a DNA fragment of 1521 base pairs encoding a C-terminal polyhistidine tag and the amino acid sequence of from Glu234 to Leu741 of human GnT-V, obtained by cutting a GnT-VΔ188 plasmid with EcoRI and EagI, into the EcoRI-EagI site of a transfer vector pAcGP67-A (manufactured by PharMingen). A transfer plasmid having a GnT-VΔ436 gene was produced by ligating a DNA fragment of 912 base pairs encoding a C-terminal polyhistidine tag and the amino acid sequence of from Ile437 to Leu741 of human GnT-V, obtained by cutting a GnT-VΔ188 plasmid with EcoRV and EagI, into the EcoRV-EagI site of a transfer vector pAcGP67-A. For production of a recombinant Baculovirus, an insect cell Sf21 was transformed with each of the transfer plasmids obtained above according to a method known in a literature (Ikeda, Y., et al., J. Biochem. 128, 609-619 (2000)). The recombinant

glycosyltransferase derived from the transformed Sf21 cell was purified by Ni^{2+}-chelating affinity chromatography according to a method disclosed in a literature of Sasaki et al (Sasai, K., et al., Glycobiology (in press)).

[0058]

As shown in Fig. 3B, GnT-VΔ73, GnT-VΔ188 and GnT-VΔ233 variants had an HUVEC growth- and differentiation-promoting action, but GnT-VΔ436 did not have an HUVEC growth- and differentiation-promoting action. Though GnT-VΔ73 and GnT-VΔ188 had a glycosyltransferase activity, GnT-VΔ233 and GnT-VΔ436 had no glycosyltransferase activity. These results suggest that the HUVEC growth accelerating activity is present in a region corresponding to the amino acid sequence of from 234 to 436 of GnT-V and this region does not contain a region involved in a glycosyltransferase activity.

[0059]

[Example 6: Identification of basic amino acid cluster region of GnT-V inducing neovascularization]

The amino acid sequence of from 254 to 269 of human GnT-V is a sequence of Lys-Ser-Val-Arg-Gly-Lys-Gly-Lys-Gly-Gln-Lys-Arg-Lys-Arg-Lys-Lys (SEQ ID NO: 7) in which basic amino acids form a cluster, and a sequence fairly resembling this sequence is observed in the amino acid sequence of from 142 to 157 of $VEGF_{189}$ (Hauser, S. & Weich H.A., Growth Factor 9, 259-268(1993)) (see, Fig. 4). This amino acid cluster region is also kept in PIGF-2 and heparin binding type epidermal growth factor-like growth factor (HB-FGF) (see, Fig. 4), and acts as a heparin-binding motif (Hauser, S. & Weich, H.A., Growth Factor 9, 259-268 (1993)). Barillari et al. have reported that a basic

peptide having a sequence of Gly-Arg-Gly-Lys-Arg-Arg (SEQ ID NO: 10) derived from PIGF-2 releases FGF-2 from heparan sulfate proteoglycan (HSPG) on cell surface and/or extracellular matrix, to induce growth of epidermal cells (Barillari, G., et al., American J. Patho. 152, 1161-1166 (1998)).

[0060]

A basic peptide (KRKRKK peptide) composed of Lys-Arg-Lys-Arg-Lys-Lys (SEQ ID NO: 11) which is the amino acid sequence of from 264 to 269 and a non-basic control peptide (FSGGPL peptide) composed of Phe-Ser-Gly-Gly-Pro-Leu (SEQ ID NO: 12) which is the amino acid sequence of from 291 to 296, of GnT-V, were synthesized, and an influence of these peptides on growth of HUVEC was examined. The peptides were synthesized by a peptide synthesizer A432 (manufactured by Applied Biosystems), and purified by reverse phase HPLC. Then, their molecular weights and purities were confirmed by MALDI TOF-MS (Voyager-DE (registered trademark) RP; manufactured by PerSeptive Biosystems). The concentration of FGF-2 was measured by a known method (Barillari, G., et al., American J. Patho. 152, 1161-1166 (1998)). That is, HUVEC was seeded on a 12-well plate coated with collagen in an amount of 5×10^4 cells per well, and washed twice with PBS, then, the medium was substituted with MCDB 131/0.1% BSA (0.5 ml/well), and the plate was maintained at 4°C for 2 hours on a plate rotation table in the presence or absence of GnT-VΔ73, GnT-VΔ436, KRKRKK peptide or FSGGPL peptide, or in the presence of heparin. After centrifugation at 4°C and 3000 rpm for 5 minutes, the supernatant was collected, the concentration of FGF-2 in the supernatant was measured in an FGF-2 ELISA system (manufactured by R&D

Systems) according to a manual of this system.

[0061]

As described above, various deletion variants of GnT-V and synthetic peptides were added at 4°C to culture solution of HUVEC, and the amount of FGF-2 released from HSPG on HUVEC was measured. As a result, as shown in Fig. 5, GnT-VΔ73 and KRKRKK peptide released FGF-2, but GnT-VΔ436 and FSGGPL peptide did not affect release of FGF-2. Like GnT-VΔ73, also GnT-VΔ188 and GnT-VΔ233 released FGF-2. Similarly, heparin, which is known to release the HSPG binding molecule by competition with a heparin binding site of the HSPG binding molecule (Biard, A., et al., Proc. Natl. Acad. Sci. USA 85, 2324-2328 (1988)), induced release of FGF-2. Phosphorylation of an FGF receptor on HUVEC by stimulation of released FGF-2 was also confirmed. As shown in Fig. 6, the KRKRKK peptide accelerated growth of HUVEC at the same degree as GnT-VΔ73, but this effect was completely suppressed by an anti-FGF-2 neutralization antibody added simultaneously. These results suggest that a basic amino acid cluster region of GnT-V is sufficient for an HUVEC growth accelerating activity, and a GnT-V protein releases FGF-2 from HSPG on an endothelial cell by the action of its own basic region, to accelerate neovascularization.

[0062]

[Example 7: *In vivo* neovascularization induced by GnT-V protein]

Induction of neovascularization by GnT-V was confirmed also by other *in vitro* neovascularization assays such as a capillary-like tube formation assay (Ashoton, A.W., et al., J. Biol. Chem. 274, 35562-35570 (1999)) and a migration assay (Zeng,

H., et al., J. Biol. Chem. 276, 3271-3279 (2001)) using HUVEC. For confirming ex-vivo neovascularization activity of GnT-V, a CAM assay using a GnT-VΔ73 protein was conducted. The GnT-VΔ73 induced neovascularization of chicken microvessels like FGF-2, and the KRKRKK peptide induced neovascularization likewise, but induction of neovascularization by either of GnT-VΔ73 and KRKRKK peptide was inhibited by treatment with an anti-FGF-2 neutralization antibody. In contrast, GnT-VΔ436 and FSGGPL peptide did not have a neovascularization activity. These results indicate that secretory type GnT-V and the KRKRKK peptide derived from GnT-V induce neovascularization via the action of FGF-2, and that the basic region of GnT-V causes release of FGF-2 from HSPG on an endothelial cell, in view of the results of the HUVEC differentiation and growth assay.

[0063]

[Effect of the Invention]

The present invention provides a protein having a neovascularization action, and a neovascularization accelerator containing this protein. This neovascularization accelerator is effective for wound healing or, for prevention and/or treatment of diseases related to arteriosclerosis, thrombosis, aneurysm, and vascular obstruction.

Further, according to the present invention, a neovascularization action can be suppressed by suppressing conversion of mature transmembrane type GnT-V into secretory type GnT-V. A neovascularization action can be suppressed also by suppressing expression of GnT-V and suppressing binding of secretory type GnT-V to heparan sulfate proteoglycan. Such substances suppressing a neovascularization action are

effective for prevention and/or treatment of diseases caused by neovascularization, typically including cancer metastasis and the like.

Furthermore, by use of an antibody to the above-mentioned peptide or protein containing a basic amino acid cluster region of GnT-V, the presence or absence or the amount of the above-mentioned peptide or protein in a test sample can be determined, and for example, a possibility of cancer metastasis can be found.

[0064]

[Sequence Listing]

<110> SUNTORY LIMITED

<120> Glycosyltransferase GnT-V having angiogenic activity

<130> DS07J617

<160> 12

<210> 1

<211> 5

<212> PRT

<213> Homo sapiens

<223>

<400> 1

Thr Pro Trp Gly Lys

1 5

<210> 2

<211> 6

<212> PRT

<213> Homo sapiens

<223>

<400> 2

Asn Ile Pro Ser Tyr Val
1 5

<210> 3

<211> 17

<212> PRT

<213> Homo sapiens

<223>

<400> 3

Val Leu Asp Ser Phe Gly Thr Glu Pro Glu Phe Asn His Ala Asn Tyr
1 5 10 15

Ala

<210> 4

<211> 6

<212> PRT

<213> Homo sapiens

<223>

<400> 4

Asp Leu Gln Phe Leu Leu
1 5

<210> 5

<211> 7

<212> PRT

<213> Homo sapiens

<223>

<400> 5

Asn Thr Asp Phe Phe Ile Gly
1 5

<210> 6

<211> 2095

<212> cDNA

<213> Homo sapiens

<223>

<400> 6

CCGGCTGAAG CATCAGAATG GAAGTGAGGA AAGGCAACCA GCTGACACAG GAGCCAGAGT 60

GAGACCAGCA GACTCTCACA CTCAACCTAC ACCATGAATT TGTGTCTATC TTCTACGCGT 120

TAAGAGCCAA GGACAGGTGA AGTTGCCAGA GAGCA ATG GCT CTC TTC ACT CCG 173
Met Ala Leu Phe Thr Pro
1 5

TGG AAG TTG TCC TCT CAG AAG CTG GGC TTT TTC CTG GTG ACT TTT GGC 221
Trp Lys Leu Ser Ser Gln Lys Leu Gly Phe Phe Leu Val Thr Phe Gly
10 15 20

TTC ATT TGG GGT ATG ATG CTT CTG CAC TTT ACC ATC CAG CAG CGA ACT 269
Phe Ile Trp Gly Met Met Leu Leu His Phe Thr Ile Gln Gln Arg Thr
25 30 35

CAG CCT GAA AGC AGC TCC ATG CTG CGC GAG CAG ATC CTG GAC CTC AGC 317
Gln Pro Glu Ser Ser Ser Met Leu Arg Glu Gln Ile Leu Asp Leu Ser

40 45 50

AAA AGG TAC ATC AAG GCA CTG GCA GAA GAA AAC AGG AAT GTG GTG GAT 365
Lys Arg Tyr Ile Lys Ala Leu Ala Glu Glu Asn Arg Asn Val Val Asp
55 60 65 70

GGG CCA TAC GCT GGA GTC ATG ACA GCT TAT GAT CTG AAG AAA ACC CTT 413
Gly Pro Tyr Ala Gly Val Met Thr Ala Tyr Asp Leu Lys Lys Thr Leu
75 80 85

GCT GTG TTA TTA GAT AAC ATT TTG CAG CGC ATT GGC AAG TTG GAG TCG 461
Ala Val Leu Leu Asp Asn Ile Leu Gln Arg Ile Gly Lys Leu Glu Ser
90 95 100

AAG GTG GAC AAT CTT GTT GTC AAT GGC ACC GGA ACA AAC TCA ACC AAC 509
Lys Val Asp Asn Leu Val Val Asn Gly Thr Gly Thr Asn Ser Thr Asn
105 110 115

TCC ACT ACA GCT GTT CCC AGC TTG GTT GCA CTT GAG AAA ATT AAT GTG 557
Ser Thr Thr Ala Val Pro Ser Leu Val Ala Leu Glu Lys Ile Asn Val
120 125 130

GCA GAT ATC ATT AAC GGA GCT CAA GAA AAA TGT GTA TTG CCT CCT ATG 605
Ala Asp Ile Ile Asn Gly Ala Gln Glu Lys Cys Val Leu Pro Pro Met
135 140 145 150

GAC GGC TAC CCT CAC TGT GAG GGA AAG ATC AAG TGG ATG AAA GAC ATG 653
Asp Gly Tyr Pro His Cys Glu Gly Lys Ile Lys Trp Met Lys Asp Met
155 160 165

TGG CGT TCA GAT CCC TGC TAC GCA GAC TAT GGA GTG GAT GGA TCC ACC 701
Trp Arg Ser Asp Pro Cys Tyr Ala Asp Tyr Gly Val Asp Gly Ser Thr
170 175 180

TGC TCT TTT TTT ATT TAC CTC AGT GAG GTT GAA AAT TGG TGT CCT CAT 749
Cys Ser Phe Phe Ile Tyr Leu Ser Glu Val Glu Asn Trp Cys Pro His
185 190 195

TTA CCT TGG AGA GCA AAA AAT CCC TAC GAA GAA GCT GAT CAT AAT TCA 797
Leu Pro Trp Arg Ala Lys Asn Pro Tyr Glu Glu Ala Asp His Asn Ser
200 205 210

TTG GCG GAA ATT CGT ACA GAT TTT AAT ATT CTC TAC AGT ATG ATG AAA 845
Leu Ala Glu Ile Arg Thr Asp Phe Asn Ile Leu Tyr Ser Met Met Lys
215 220 225 230

AAG CAT GAA GAA TTC CGG TGG ATG AGA CTA CGG ATC CGG CGA ATG GCT 893
Lys His Glu Glu Phe Arg Trp Met Arg Leu Arg Ile Arg Arg Met Ala
235 240 245

GAC GCA TGG ATC CAA GCA ATC AAG TCC CTG GCA GAA AAG CAG AAC CTT 941
Asp Ala Trp Ile Gln Ala Ile Lys Ser Leu Ala Glu Lys Gln Asn Leu
250 255 260

GAA AAG AGA AAG CGG AAG AAA GTC CTC GTT CAC CTG GGA CTC CTG ACC 989
Glu Lys Arg Lys Arg Lys Lys Val Leu Val His Leu Gly Leu Leu Thr
265 270 275

AAG GAA TCT GGA TTT AAG ATT GCA GAG ACA GCT TTC AGT GGT GGC CCT 1037
Lys Glu Ser Gly Phe Lys Ile Ala Glu Thr Ala Phe Ser Gly Gly Pro
280 285 290

CTT GGT GAA TTA GTT CAA TGG AGT GAT TTA ATT ACA TCT CTG TAC TTA 1085
Leu Gly Glu Leu Val Gln Trp Ser Asp Leu Ile Thr Ser Leu Tyr Leu
295 300 305 310

CTG GGC CAT GAC ATT AGG ATT TCA GCT TCA CTG GCT GAG CTC AAG GAA 1133
Leu Gly His Asp Ile Arg Ile Ser Ala Ser Leu Ala Glu Leu Lys Glu
315 320 325

ATC ATG AAG AAG GTT GTA GGA AAC CGA TCT GGC TGC CCA ACT GTA GGA 1181
Ile Met Lys Lys Val Val Gly Asn Arg Ser Gly Cys Pro Thr Val Gly
330 335 340

GAC AGA ATT GTT GAG CTC ATT TAC ATT GAT ATT GTA GGA CTT GCT CAA 1229
Asp Arg Ile Val Glu Leu Ile Tyr Ile Asp Ile Val Gly Leu Ala Gln
345 350 355

TTC AAG AAA ACT CTT GGA CCA TCC TGG GTT CAT TAC CAG TGC ATG CTC 1277
Phe Lys Lys Thr Leu Gly Pro Ser Trp Val His Tyr Gln Cys Met Leu
360 365 370

CGA GTC CTT GAT TCA TTT GGT ACT GAA CCC GAA TTT AAT CAT GCA AAT 1325
Arg Val Leu Asp Ser Phe Gly Thr Glu Pro Glu Phe Asn His Ala Asn
375 380 385 390

TAT GCC CAA TCG AAA GGC CAC AAG ACC CCT TGG GGA AAA TGG AAT CTG 1373

Tyr Ala Gln Ser Lys Gly His Lys Thr Pro Trp Gly Lys Trp Asn Leu
395 400 405

AAC CCT CAG CAG TTT TAT ACC ATG TTC CCT CAT ACC CCA GAC AAC AGC 1421
Asn Pro Gln Gln Phe Tyr Thr Met Phe Pro His Thr Pro Asp Asn Ser
410 415 420

TTT CTG GGG TTT GTG GTT GAG CAG CAC CTG AAC TCC AGT GAT ATC CAC 1469
Phe Leu Gly Phe Val Val Glu Gln His Leu Asn Ser Ser Asp Ile His
425 430 435

CAC ATT AAT GAA ATC AAA AGG CAG AAC CAG TCC CTT GTG TAT GGC AAA 1517
His Ile Asn Glu Ile Lys Arg Gln Asn Gln Ser Leu Val Tyr Gly Lys
440 445 450

GTG GAT AGC TTC TGG AAG AAT AAG AAG ATC TAC TTG GAC ATT ATT CAC 1565
Val Asp Ser Phe Trp Lys Asn Lys Lys Ile Tyr Leu Asp Ile Ile His
455 460 465 470

ACA TAC ATG GAA GTG CAT GCA ACT GTT TAT GGC TCC AGC ACA AAG AAT 1613
Thr Tyr Met Glu Val His Ala Thr Val Tyr Gly Ser Ser Thr Lys Asn
475 480 485

ATT CCC AGT TAC GTG AAA AAC CAT GGT ATC CTC AGT GGA CGG GAC CTG 1661
Ile Pro Ser Tyr Val Lys Asn His Gly Ile Leu Ser Gly Arg Asp Leu
490 495 500

CAG TTC CTT CTT CGA GAA ACC AAG TTG TTT GTT GGA CTT GGG TTC CCT 1709
Gln Phe Leu Leu Arg Glu Thr Lys Leu Phe Val Gly Leu Gly Phe Pro

505 510 515

TAC GAG GGC CCA GCT CCC CTG GAA GCT ATC GCA AAT GGA TGT GCT TTT 1757
Tyr Glu Gly Pro Ala Pro Leu Glu Ala Ile Ala Asn Gly Cys Ala Phe
520 525 530

CTG AAT CCC AAG TTC AAC CCA CCC AAA AGC AGC AAA AAC ACA GAC TTT 1805
Leu Asn Pro Lys Phe Asn Pro Pro Lys Ser Ser Lys Asn Thr Asp Phe
535 540 545 550

TTC ATT GGC AAG CCA ACT CTG AGA GAG CTG ACA TCC CAG CAT CCT TAC 1853
Phe Ile Gly Lys Pro Thr Leu Arg Glu Leu Thr Ser Gln His Pro Tyr
555 560 565

GCT GAA GTT TTC ATC GGG CGG CCA CAT GTG TGG ACT GTT GAC CTC AAC 1901
Ala Glu Val Phe Ile Gly Arg Pro His Val Trp Thr Val Asp Leu Asn
570 575 580

AAT CAG GAG GAA GTA GAG GAT GCA GTG AAA GCA ATT TTA AAT CAG AAG 1949
Asn Gln Glu Glu Val Glu Asp Ala Val Lys Ala Ile Leu Asn Gln Lys
585 590 595

ATT GAG CCA TAC ATG CCA TAT GAA TTT ACG TGC GAG GGG ATG CTA CAG 1997
Ile Glu Pro Tyr Met Pro Tyr Glu Phe Thr Cys Glu Gly Met Leu Gln
600 605 610

AGA ATC AAT GCT TTC ATT GAA AAA CAG GAC TTC TGC CAT GGG CAA GTG 2045
Arg Ile Asn Ala Phe Ile Glu Lys Gln Asp Phe Cys His Gly Gln Val
615 620 625 630

ATG TGG CCA CCC CTC AGC GCC CTA CAG GTC AAG CTT GCT GAG CCC GGG 2093
Met Trp Pro Pro Leu Ser Ala Leu Gln Val Lys Leu Ala Glu Pro Gly
635 640 645

CC 2095

<210> 7
<211> 16
<212> PRT
<213> Homo sapiens
<223>
<400> 7
Lys Ser Leu Ala Glu Lys Gln Asn Leu Glu Lys Arg Lys Arg Lys Lys
1 5 10 15

<210> 8
<211> 24
<212> cDNA
<213> Artificial Sequence
<223>
<400> 8
GGGAGTGAGG ATGATGTAGG GAAG 24

<210> 9
<211> 24
<212> cDNA
<213> Artificial Sequence
<223>

<400> 9

ATGGGGCAGA GGAACTTACG TTAT 24

<210> 10

<211> 6

<212> PRT

<213> Homo sapiens

<223>

<400> 10

Gly Arg Gly Lys Arg Arg

1 5

<210> 11

<211> 6

<212> PRT

<213> Homo sapiens

<223> KRKRKK peptide

<400> 11

Lys Arg Lys Arg Lys Lys

1 5

<210> 12

<211> 6

<212> PRT

<213> Homo sapiens

<223> FSGGPL peptide

<400> 12

Phe Ser Gly Gly Pro Leu

1 5

[Brief Description of the Drawings]

[Fig. 1] is a view showing the differentiation and growth of HUVEC treated with culture solution of each cell, in terms of the amount of incorporation of [^{3}H]-thymidine as an index. CRT is a normal fresh medium used for culture of HUVEC.

[Fig. 2] is a view showing a relation between the addition amount of GnT-VΔ73 and the differentiation and growth of HUVEC.

[Fig. 3] Fig. 3A is a schematic view of an amino acid sequence of each deletion variant of GnT-V. Fig. 3 B is a view showing an HUVEC differentiation- and growth-promoting action by each deletion variant of GnT-V.

[Fig. 4] is a view showing similarity between an amino acid sequence of a basic cluster region of GnT-V and amino acid sequences of $VEGF_{189}$, PlGF-2 and HB-EGF.

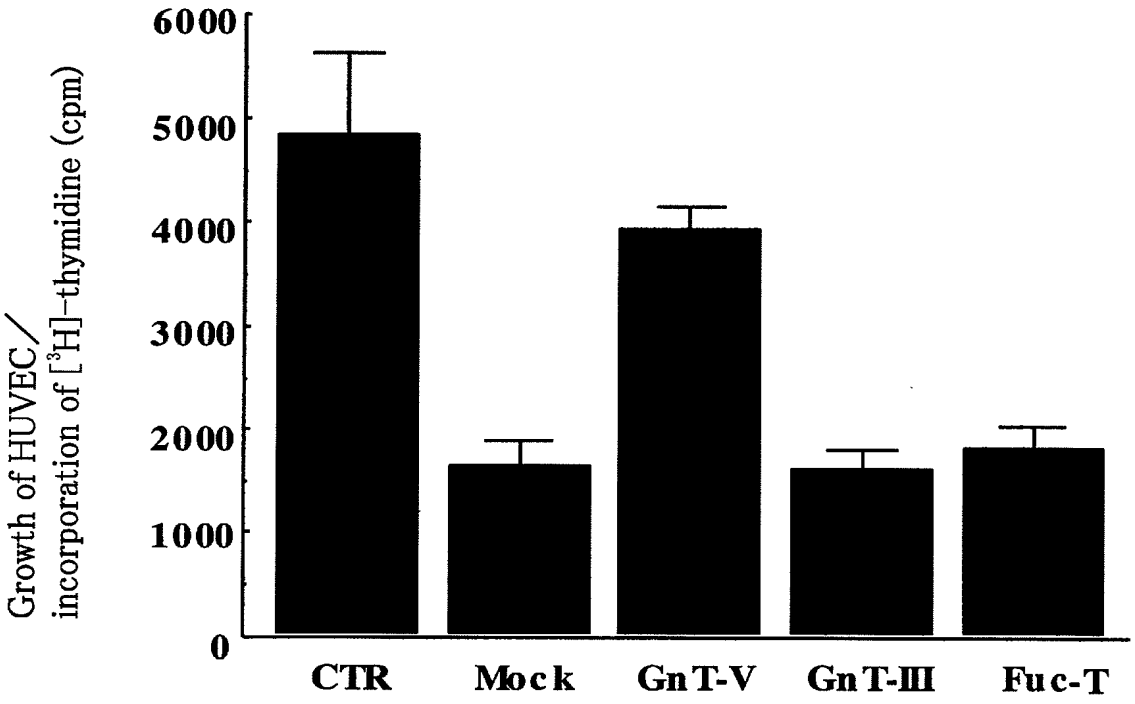
[Fig. 5] is a view showing the released amounts of FGF-2 by various deletion variants of GnT-V and synthetic peptides.

[Fig. 6] is a view showing an HUVEC differentiation- and growth-promoting action by various deletion variants of GnT-V and synthetic peptides.

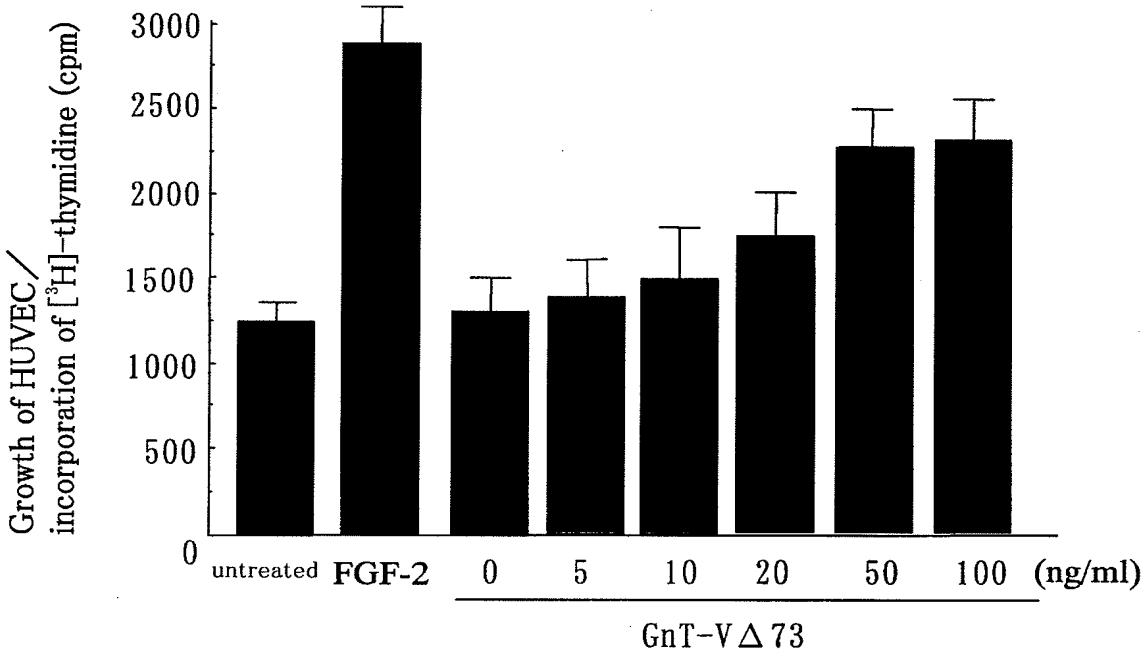
[Fig. 7] is a schematic view showing induction of cancer neovascularization by GnT-V. Secretory type GnT-V containing a basic amino acid cluster region binds, in competition with FGF-2, to HSPG on the surface of a cell, and resultantly, FGF-2 is released, to stimulate a FGF-2 receptor on the target cell.

[Name of Document] Drawings

[Fig. 1]

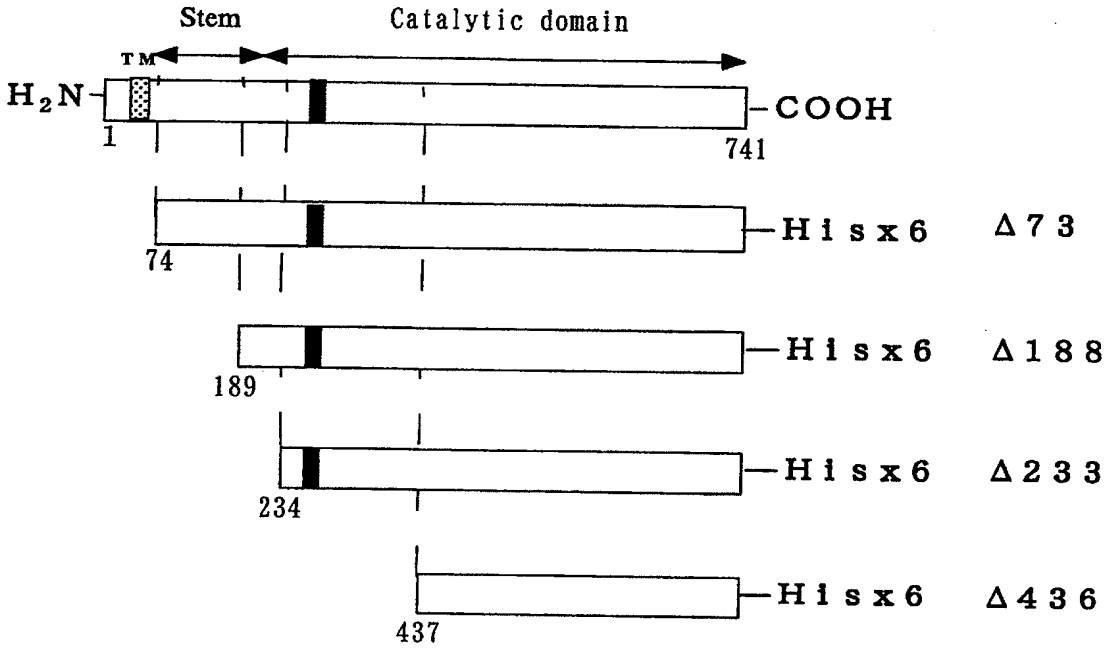


[Fig. 2]

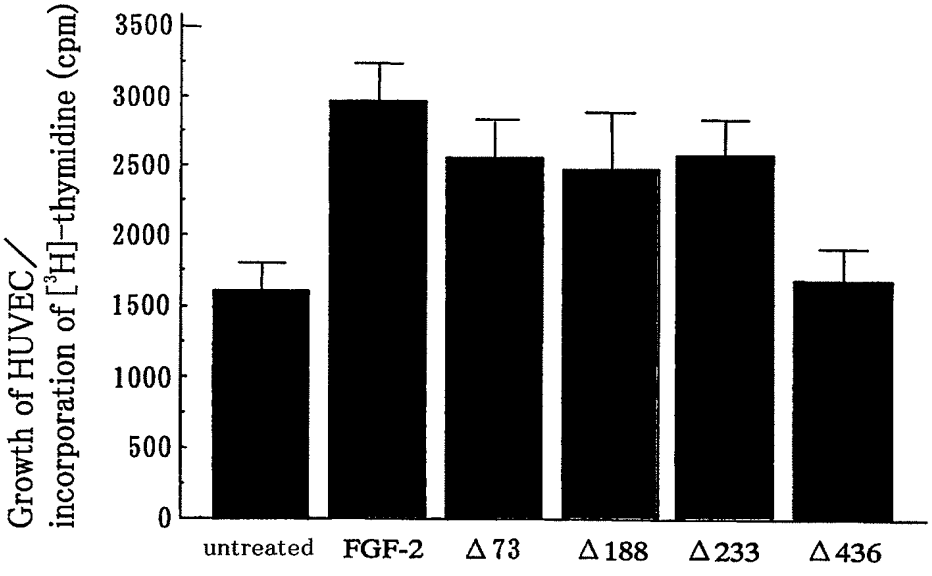


[Fig. 3]

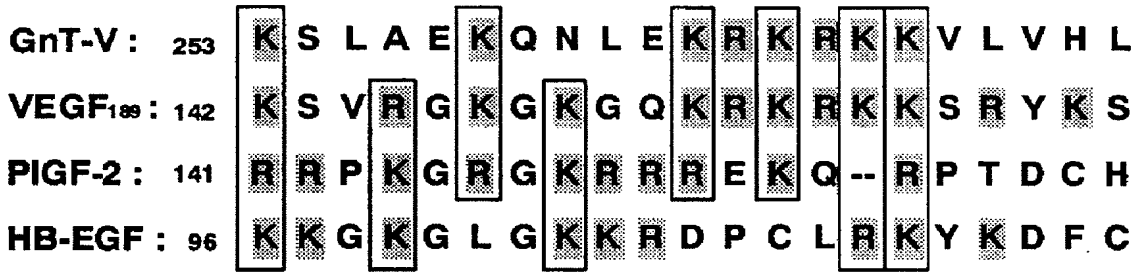
(A)


Stem
Catalytic domain
TM
H2N
COOH
1
741
His x6
Δ73
74
His x6
Δ188
189
His x6
Δ233
234
His x6
Δ436
437

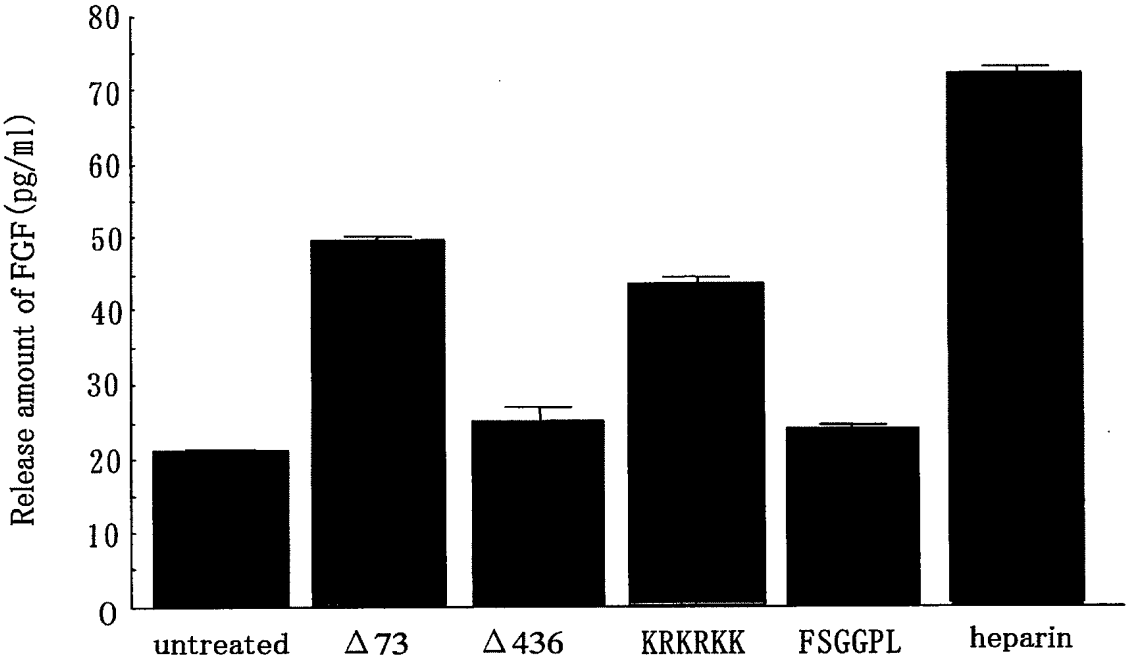
(B)


Growth of HUVEC/
incorporation of [3H]-thymidine (cpm)
3500
3000
2500
2000
1500
1000
500
0
untreated
FGF-2
Δ73
Δ188
Δ233
Δ436

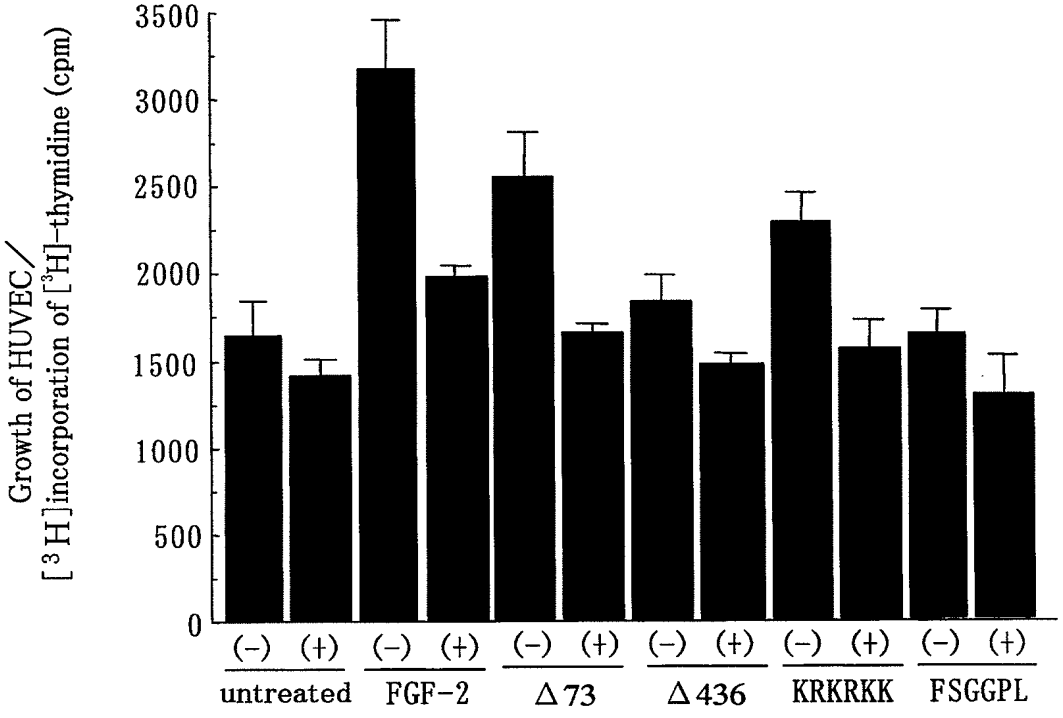
[Fig. 4]


GnT-V : 253 K S L A E K Q N L E K R K R K K V L V H L
VEGF189 : 142 K S V R G K G K G Q K R K R K K S R Y K S
PlGF-2 : 141 R R P K G R G K R R R E K Q -- R P T D C H
HB-EGF : 96 K K G K G L G K K R D P C L R K Y K D F C

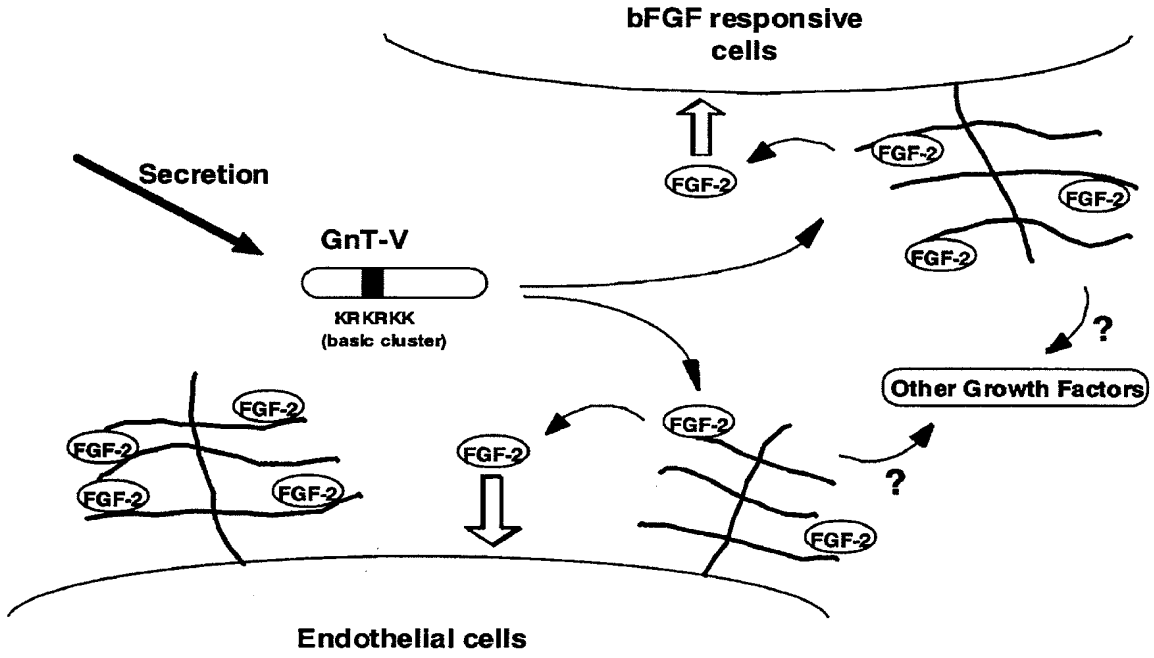
[Fig. 5]


Release amount of FGF (pg/ml)
0
10
20
30
40
50
60
70
80
untreated
Δ73
Δ436
KRKRKK
FSGGPL
heparin

[Fig. 6]


Growth of HUVEC/
[3H]incorporation of [3H]-thymidine (cpm)
3500
3000
2500
2000
1500
1000
500
0
(−) (+) (−) (+) (−) (+) (−) (+) (−) (+) (−) (+)
untreated
FGF-2
Δ73
Δ436
KRKRKK
FSGGPL

[Fig. 7]


bFGF responsive cells
Secretion
GnT-V
KRKRKK
(basic cluster)
FGF-2
Other Growth Factors
?
Endothelial cells

[Name of Document] Abstract

[Abstract]

[Problem]

An object of the present invention is to provide a new therapeutic target relating to cancer metastasis and growth which are most important problems in cancer therapy by clarifying a role played by a glycosyltransferase GnT-V on cancer metastasis and growth.

[Solution]

A peptide or protein having a neovascularization action and containing a basic amino acid cluster region of β1,6-N-acetylglucosaminyltransferase.

[Chosen drawing]

None